Research Article

Variability Analysis in Ginger (*Zingiber officinale* Rosc.) Somaclones Using ISSR Marker

S S Gavande*, M R Shylaja, and P A Nazeem

Centre for Plant Biotechnology and Molecular Biology, Kerala Agricultural University, Thrissur -680656, Kerala, India

Abstract

Thirteen groups of ginger somaclones which include 180 plants in total along with two source parent cultivars (Maran and Rio-de-Janeiro) were evaluated using ISSR marker systems. In ISSR assay, twelve selected primers produced 122 amplicons, 32 were polymorphic with an average of 2.66 polymorphic bands/ primer and a polymorphism percentage of 26.23. The dendrograms generated based on ISSR profiles grouped the somaclones into two separate clusters. ISSR marker systems showed that somaclones derived from cultivar Maran exhibited more variability than Rio-de-Janeiro. In groupwise variability analysis using bulked DNA, the groups RC20 Gy and RSe10 Gy recorded higher variability from source parent cultivar. The somaclone RC2Kr1031 of the callus regenerants and RSe1Kr1052 of the somatic embryo regenerants showed more variability exhibiting 59 and 53 per cent respectively from the source parent cultivar Rio-de-Janeiro.

Keywords: Bulked DNA; ginger; ISSR; somaclonal variation

***Correspondence**
Author: S S Gavande
Email:
sharda.gavande26@gmail.com

Introduction

Ginger (*Zingiber officinale* Rosc.), an important spice crop grown in India, is much valued for its flavour and medicinal properties. Crop improvement through selection and hybridization are not effective in ginger due to lack of variability and absence of natural seed set. The earlier crop improvement programmes were hence focussed on mutation breeding using γ rays and ethyl methyl sulfonate (EMS). The mutants thus isolated were low yielders and the effect of mutagen treatment vanished in subsequent generations [1, 2, 3]. Hence investigations were made to induce variability in ginger through biotechnological tools like *in vitro* pollination and exploitation of somaclonal variation.

Currently, molecular marker techniques are widely employed to detect and assess somaclonal variation in several crops as they are stable, detectable in all tissues and are not confounded by environment, pleiotropic and epistatic effects. Inter Simple Sequence Repeats **(**ISSR) markers can be used to assess the genetic stability of micropropogated plants like ginger [4, 5]. In addition, Inter Simple Sequence Repeats are considered useful in gene mapping studies [6, 7,8], diversity analysis in many crops including ginger [9] and somaclonal variation [10]. The variability analysis in the thirteen groups of ginger somaclones (180 Nos.) of two cultivars Maran and Rio-de-Janeiro using ISSR marker system was attempted in the present study.

Materials and methods

The somaclones from bud culture of the two cultivars Maran and Rio-de-Janeiro were planted out for rhizome formation in 1999-2000 after passing through ten to twelve subculture cycles and the clones were evaluated for yield, quality and tolerance to soft rot and bacterial wilt diseases. Preliminary field evaluation, advanced variety trials, onfarm evaluation multilocational tests, large scale demonstration of selected clones were undertaken during the period from 2002 to 2010 and two selected superior somaclones from bud culture regenerants were released under the name Athira and Karthika during 2010. The somaclones regenerated through indirect methods and regenerants from irradiated organogenic and embryogenic calli of the two cultivars were planted out for rhizome formation in 2004. Preliminary yield evaluation and evaluation for soft rot and bacterial wilt diseases in the clones were completed during 2006 to 2010. The evaluation of the clones at molecular level and variability analysis using ISSR marker system was attempted in the present study. Thirteen groups of somaclones which include 180 plants in total (seven groups in Maran and six groups in Rio-de-Janeiro) based on mode of regeneration along with two source parent cultivars were subjected to ISSR analysis.

DNA was extracted from somaclones using CTAB method [11] and Sigma's GenElute^{TM} Plant Genomic DNA Miniprep kit. The quality and quantity of genomic DNA was estimated using NanoDrop^{R} ND-1000 spectrophometer and agarose gel electrophoresis. The somaclones were grouped as per genotype and mode of regeneration. DNA extracted from individual 180 somaclones was bulked as per the procedure reported by [12]. Bulked DNA samples of the thirteen groups of somaclones (seven in Maran and six in Rio-de-Janeiro) along with two source parent cultivars were amplified using selected ISSR primers. The genotype and mode of regeneration exhibiting more variability was focused for further indepth investigations, using individual DNA of each somaclone.

A total of 30 ISSR primers were screened and those primers which gave good amplification products were selected for further analysis. The DNA amplification was performed in a thermal cycler in 20 µl reaction mixture consisting of 2 µl 10X *Taq* assay buffer B with 2 µl MgCl2, 1.5 µl dNTPs, 10pM of single random primer, 30 ng template DNA and 0.4 µl of *Taq* DNA polymerase. Reactions were programmed for one cycle at 93°C for one min., 40 cycles repeated running at 93°C for one min., 37°C for one min. and 72°C for two min. followed by one cycle at 72°C for eight min. The amplification products were resolved by electrophoresis in two percent agarose gel using 1X TAE buffer and visualized under UV light. The ISSR profiles of the thirteen groups of somaclones for different primers were scored based on the presence (1) or absence (0) of bands. The data were analysed using NTSYS pc version 2.02i [13]. The genetic similarity was estimated by Jaccard's coefficient and dendrogram was generated by using Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Resolving power (Rp) of a primer was calculated as the sum of 'band informativeness' of all the bands produced by the primer. Band information (Ib) is = 1-[2(0.5 – p)], where p is the proportion of the somaclones containing the bands. Resolving power of the primer is represented as: Rp= Σ Ib. PIC value was calculated as PIC = 1- Σ pi^2, where *pi* was the frequency of the i^{th} allele.

Results and Discussion

Variability analysis in ginger somaclones with selected ISSR primers:

Out of the 30 ISSR primers screened, twelve gave good amplification (**Table 1**). ISSR analysis using twelve selected primers produced produced a total of 122 amplicons of which 32 were polymorphic giving a polymorphism of 26.23 per cent with an average of 10.16 markers per primer and a mean of 2.66 polymorphic bands per primer (**Table 2**). [12] reported a total 66 bands were produced with an average of 8.3 bands per primer when they assessed stability of mango ginger through RAPD and ISSR markers. The polymorphism percentage ranged from 12.5 to 40 in the selected primers. The highest polymorphism percentage was recorded by the primer ISSR 05 (40) followed by UBC 835 (30.76), UBC 834 (36.36) and ISSR 06 (33.33).

ISSR assay showed variation between somaclones derived from two ginger cultivars (Maran and Rio-de-Janeiro). Among selected ISSR primers, four primers (UBC 835, SPS 03, ISSR 05 and ISSR 06) produced unique amplicon which was present only in the Maran somaclones.

Table 1 Details of selected ISSR primers

Sl. No.	Name of Primer	Sequence	Annealing temperature (°C)
1	UBC 834	5'AGAGAGAGAGAGAGAGYT3'	45
2	UBC 835	5'AGAGAGAGAGAGAGAGY3'	43
3	UBC 840	5'GAGAGAGAGAGAGAGAYT3'	45
4	UBC 844	5'CTCTCTCTCTCTCTCTRC3'	47
5	SPS-03	5'GACAGACAGACAGACA3'	43
6	ISSR-04	5'ACACACACACACACACC3'	42
7	ISSR-05	5'CTCTCTCTCTCTCTTG3'	42
8	ISSR-06	5'GAGAGAGAGAGAGAGAC3'	47
9	ISSR-08	5'GAGAGAGAGAGAGAGAT3'	45
10	ISSR-09	5'CTCTCTCTCTCTCTCTG3'	42
11	ISSR-10	5'ACACACACACACACACG3'	47
12	ISSR-15	5'TCCTCCTCCTCCTCC3'	42

The primer UBC 840 produced an amplicon which was present only in Rio-de-Janeiro group. So the ISSR primers also could be utilized for identification of Maran and Rio-de-Janeiro cultivars.

The polymorphism information content (PIC) of selected primers ranged from 0.69 (ISSR 06) to 0.92 (ISSR 04 and UBC 835) with an average of 0.87. ISSR primers recorded Resolving power (Rp) values ranged from 11.7 (UBC 844) and 25.5 (ISSR 04) with an average 19.13.

Table 2 Amplification pattern in ginger somaclones with selected ISSR primers

Sr. No.	Primer	Total no. of amplicons	No. of polymorphic amplicons	No. of monomorphic amplicons	Size of amplicon (range-bp)	Polymorphism (%)
1	UBC 834	11	4	7	200-1000	36.36
2	UBC 835	13	4	9	200-900	30.76
3	UBC 840	11	4	7	200-1100	36.36
4	UBC 844	6	1	5	300-1000	16.66
5	SPS-03	11	3	8	350-1000	27.27
6	ISSR-04	13	2	11	200-1100	15.38
7	ISSR-05	10	4	6	350-1100	40
8	ISSR-06	9	3	6	100-800	33.33
9	ISSR-08	11	2	9	300-1000	18.18
10	ISSR-09	8	1	7	200-1400	12.5
11	ISSR-10	11	2	9	200-900	18.18
12	ISSR-15	8	2	6	400-1100	25
Total		122	32	90		26.23
Average		10.16	2.66	7.5		

The dendrogram generated using NTSYS grouped the somaclones into two main clusters. Cluster I includes eleven groups of somaclones somaclones (MB, MC, MSe, MC10 Gy, MC20 Gy, MSe10 Gy, MSe20 Gy, RB, RC, RSe, RC10 Gy) and two source parent cultivars. Rio-de-Janeiro regenerants from calli irradiated with 20 Gy and somatic embryo regenerants from embryogenic calli irradiated with 10 Gy formed the second main cluster. Cluster I was divided into two major sub clusters. Somaclones of cultivar Maran were grouped in first sub cluster and somaclones of Rio-de-Janeiro were grouped in second sub cluster (**Figure 1**). A genetic similarity matrix of different groups of ginger somaclones based on the proportion of shared ISSR fragments was also generated. The pairwise similarity coefficient values varied between 0.7603 and 1.0000 in the present investigation.

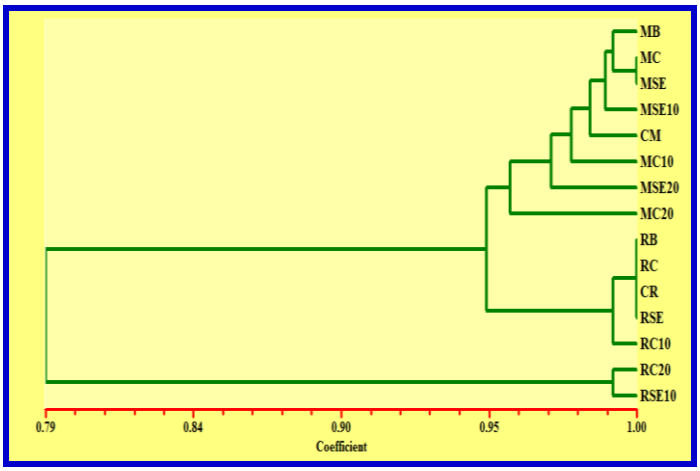

Figure 1 Dendrogram generated with ISSR profile in different groups of ginger somaclones

The extent of variability in somaclones from the source parent cultivar Maran was found more in MC20 Gy (5.8%) followed by MSe20 (4.1%), MC10 Gy (3.3%) and MSe10 Gy (2.5%). However, callus and somatic embryo regenerants showed less variability. In Rio-de-Janeiro somaclones the highest variability was recorded in RC20 Gy (19%) followed by RSe10 Gy (18%). However, bud, callus and somatic embryo regenerants were found similar to source parent cultivar.

Variability analysis in ginger somaclones of groups RC20 Gy and RSe10 Gy using one ISSR primer

Initially, amplification of bulked DNA samples from each of the thirteen groups of ginger somaclones and their source parent cultivars was carried out using selected primers of ISSR marker system. Amplification of thirteen groups of ginger somaclones and their source parent cultivars could reveal noticeable variability in RC20 Gy and RSe10 Gy among all the groups of somaclones studied. This may be due to the mode of regeneration and effect of γ irradiation on *in vitro* multiplication.

The groupwise variability analysis using bulked DNA gave an indication of the extent of variability of the group from source parent cultivars. Hence RC20 Gy and RSe10 Gy groups were focused for further investigations. DNA from each of these twelve somaclones (one of RC20 Gy and eleven of RSe10 Gy groups), were used for further variability analysis.

ISSR primer (ISSR 05) which recorded highest polymorphism was used to amplify the DNA of individual somaclones in RC20 Gy and RSe10 Gy groups along with Rio-de-Janeiro source parent cultivar.

The number of polymorphic amplicons detected by ISSR 05 primer was 6. Variability exhibited in plantwise analysis using the three selected primers was thus very high as compared to groupwise analysis. Similar observations were reported by Fu *et al*. (2003) when they assessed effectiveness of several bulking strategies in detecting RAPD variations in flax (*Linum usitatissimum* L.). In the study they observed that about 30 per cent of the polymorphic RAPD loci observed in plant-by-plant analysis were undetected in the bulked samples of the same accession.

Using NTSYS, individual ginger somaclones were grouped into two main clusters. From individual somaclones analysis five somaclones exhibiting more variability from source parent cultivar could be isolated (37% to 59%). Two somaclones RC2Kr1031 of the callus regenerants and RSe1Kr1052 of the somatic embryo regenerants showed more variability exhibiting 59 and 53 per cent variability respectively from source parent cultivar Rio-de-Janeiro (**Figure 2**).

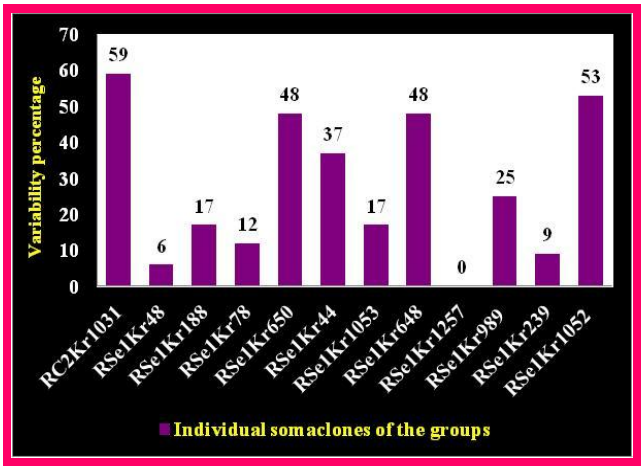

Figure 2 Variability in individual somaclones of the group RC20 Gy and RSe10 Gy

The mutants which exhibited more variability from source parent cultivar could be further evaluated for their desirable traits. The groups of somaclones which exhibited more variability in the present study were due to the absence of amplicons in the various molecular marker systems analysed. This may be due to the chromosome aberration and rearrangements, DNA methylation or histone modification as explained in somaclones and mutants by several workers [14, 15].

Conclusions

In the crops like ginger where natural variability is less, the present investigation could broaden the genetic base.
From the present investigations the following conclusions could be drawn:

Molecular marker techniques could be employed for the assessing the variability in ginger somaclones.

- The study could identify certain specific ISSR markers for identification of Maran and Rio-de-Janeiro cultivars and also irradiated mutants from non-irradiated somaclones.
- The somaclone derived from cultivar Maran exhibited more variability than somaclones of Rio-de-Janeiro.
- Irradiated callus and somatic embryo regenerants showed more variability
- The groupwise variability analysis using bulked DNA gave an indication of the extent of variability of the group from source parent cultivars.
- The variability exhibited in plantwise analysis of the selected variable groups was found high and two plants with high variability could be selected.
- The somaclone RC2Kr1031 of the callus regenerants and RSe1Kr1052 of the somatic embryo regenerants showed higher variability exhibiting 59 and 53 per cent variability respectively from source parent cultivar Rio-de-Janeiro.

- In crops like ginger where the natural variability is very less, *in vitro* mutagenesis could be employed for widening the genetic base in ginger.

Future prospects

Molecular marker analysis of individual plants of the variable groups, use of advanced marker systems for assessment of somaclonal variation, use of more number of primers to bring out variability and more focus on *in vitro* mutagenesis for widening the genetic base in ginger are the future areas to be investigated.

Acknowledgments

The authors express heartfelt thanks to Department of Biotechnology (DBT), Government of India for the financial support for the study and Kerala Agricultural University, Thrissur, India for providing materials.

References

[1] Giridharan, M.P. (1984). Effect of gamma irradiation in ginger (Zingiber officinale Rosc.). M.Sc. (Hort.) thesis, Kerala Agricultural University, Vellanikkara, India.
[2] Dutta, A.K. and Biswas, A.K. (1985). EMS induced mitotic consequences in three rhizomatous, spice yielding plants. Chromosome Inf. Serv. 38: 25-26.
[3] Jayachandran, B.K. (1989). Induced mutations in ginger. Unpublished Ph. D thesis, Kerala Agricultural University, Kerala, India.
[4] Mohanty, S.; Panda, M.K.; Sahoo, S. and Nayak, S. (2011). Micropropagation of Zingiber rubens and assessment of genetic stability through RAPD and ISSR markers. Biologia Plant. 55(1): 16-20.
[5] Mohanty, S.; Parida, R.; Kuanar, A.; Sahoo, S. and Nayak, S. (2012). Evaluation of genetic fidelity of in vitro propagated shampoo ginger (Zingiber zerumbet L.) using DNA based markers. J. Med. Plants Res. 6(16): 3143-3147.
[6] Godwin, J.D.; Aitken, E.A.B. and Smith, L.W. (1997). Application of Inter Simple Sequence Repeat (ISSR) markers to plant genetics. Electrophoresis. 18: 1524-1528.
[7] Zietkiewicz, E.; Rafalski, A. and Labuda, D. (1994). Genome fingerprinting by inter simple sequence repeat (ISSR) anchored polymerase chain reaction amplification. Genomics. 20: 176-183.
[8] Gupta, M.; Chyi, Y.S. and Romero-Severson, J.L. (1994). Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple sequence repeats. Theor. Appl. Genet. 89: 998-1006.
[9] Kizhakkayil, J. and Sasikumar, B. (2010). Genetic diversity analysis of ginger (Zingiber officinale Rosc.) germplasm based on RAPD and ISSR markers. Scientia Horticulturae. 125: 73-76.
[10] Ngezahayo, F.; Dong, Y.S. and Liu, B. (2007). Somaclonal variation at the nucleotide sequence level in rice (Oryza sativa L.) as revealed by RAPD and ISSR markers, and by pairwise sequence analysis. J. Appl. Genet. 48(4): 329-336.
[11] Rogers, S.O. and Bendich, A.J. (1994). Extraction of total cellular DNA from plants, algae and fungi; In: Gelvin, S.B. and Schilperoort, R.A. (eds.) Plant Mol. Biol. 1: 1-8.
[12] Dulson, J.; Kott, L.S. and Ripley, V.L. (1998). Efficacy of bulked DNA samples for RAPD DNA fingerprinting of genetically complex Brassica napus cultivars. Euphytica. 102: 65-70.
[13] Rohlf, F.J. 2005. NTSYSpc Numerical Taxonomy and Multivariate Analysis System. Verson 2.2. Exeter software, New York.
[14] Larkin, P. and Scowcroft, W. (1981). Somaclonal variation - a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet. 60: 197-214.
[15] Skirvin, R.M. and Janick, J. (1996). Tissue culture induced variation in scented pelargonium spp. J. Am. Soc. hort. Sci. 101: 281-290.

Publication History
Received 12th Aug 2018
Revised 26th Sep 2018
Accepted 12th Oct 2018
Online 30th Oct 2018